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EFFECTS OF CRUDE OiL AND SOME OF ITS COMPONENTS ON YOUNG COHO AND SOCKEYE SALMON

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EFFECTS OF CRUDE OIL AND SOME OF ITS COMPONENTS ON YOUNG COHO AND SOCKEYE SALMON

by

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Project R801039 (formerly 16100FWQ)
Program Element 1BA022

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ABSTRACT

Young coho and sockeye salmon, acclimated to 30 °/oo salinity, were exposed in various ways to different amounts of crude oil from the Prudhoe Bay field. Oil poured on the surface of the water in 95 liter (25 gallon) aquaria produced significant mortalities when the oil concentration was 500 ppm or greater. Fish dipped into a crude oil film, or with a drop of oil placed directly on each gill, showed no significant mortalities. The same was true of fish force-fed crude oil at 1 g per 100 g body weight. Oil that had been exposed to air for 30 days produced no significant mortalities.

Among oil components tested for toxicity on coho salmon, aliphatic compounds were not lethal. Mono-cyclic aromatics were generally toxic, the degree of toxicity increasing with the degree of unsaturation.

It is suggested that the toxicity of these substances is brought about through alteration of cell membrane permeability, especially in the gills. This results in a rapid increase of mono-valent ions in the blood and probably also interferes with CO_2-HCO_3^- regulation.

This report submitted in fulfillment of Project R801039 (formerly 16100FWQ) by the Department of Biological Sciences, University of Alaska, Fairbanks, Alaska, under the sponsorship of the Environmental Protection Agency. Work was completed in May 1973.

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27 Abstract

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Among oil components tested for toxicity on coho salmon, aliphatic compounds were not lethal. Mono-cyclic aromatics were generally toxic, the degree of toxicity increasing with the degree of unsaturation.

It is suggested that the toxicity of these substances is brought about through alteration of cell membrane permeability, especially in the gills. This results in a rapid increase of mono-valent ions in the blood and probably also interferes with CO_2-HCO_3^- regulation. (EPA)

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SECTION I

CONCLUSIONS

Under laboratory conditions which attempted to duplicate a natural environment, as far as this could be done within the confines of 95 liter (25 gallon) aquaria, young coho and sockeye salmon are vulnerable to crude oil slicks when the amount of oil present is in the equivalent of 500 ppm or greater. Fish so exposed exhibit a typical behavior pattern, and the majority die within 96 hours. At lower concentrations of oil, the behavior pattern is less marked and mortalities are lower.

Aliphatic compounds are relatively innocuous, whether they be saturated or mono-unsaturated. By contrast, monocyclic aromatic compounds are generally toxic, and the degree of toxicity increases with increasing unsaturation.

Toxicity of unsaturated aromatic compounds probably results from alteration of cell membrane permeability, especially in the gills, by dissolution of fatty substances from them. This, in turn, destroys the ability to regulate salt balance. The monovalent ions sodium, potassium, anc chloride increase rapidly but temporarily in the blood, and probably in the tissues as well. The observed symptoms are muscle hypertension, hyperactivity, loss of equilibrium, and death. These symptoms are consistent with the thesis that ionic imbalance in blood and tissues, together with loss of control of CO_2-HCO_3^- balance, are the active mechanisms.

SECTION II

RECOMMENDATIONS

The theories presented in this report should be tested, utilizing more sophisticated instrumentation and techniques and larger animals.

We suggest that the matter of altered cell membrane permeability could be determined by perfusion techniques on excised gills. Swim bladder gases should be analyzed to determine whether any change in composition appears after exposure to aromatic substances. Blood pH and CO_2 should be measured at the same time, with the blood taken from the aorta close to the gills. This should determine whether the CO_2-HCO_3^- balance is actually disturbed.

SECTION III

INTRODUCTION

GENERAL

The discovery of oil on the North Slope of Alaska and the construction of the proposed trans-Alaska pipeline from Prudhoe Bay to Valdez present the threat of oil pollution in one of the major fisheries areas of Alaska. Until recently, Alaskan fisheries have been singularly free of this sort of pollution, but as the possibility of transporting vast quantities of oil from the port of Valdez increases, so also does the probability of oil pollution in the area. Since the fisheries are one of the most valuable industries in the state and will undoubtedly continue for a long time to occupy this position in the economy, it is necessary to investigate the effects of oil on the fisheries in order to be able to predict, ameliorate, and, hopefully, avoid altogether damage to this major source of income and employment. The present project was designed to contribute information that might be applied towards this goal, by determining the toxicity levels of crude oil and some of its components and also by attempting to determine the mechanisms of this toxicity.

The area of greatest interest from the standpoint of the relationship of oil pollution to the fisheries is Prince William Sound and its immediate environs. For this reason, laboratory conditions were designed to duplicate, as nearly as possible, the conditions in that body of water. All experiments were conducted in artificial sea water of approximately 30 $^{o}/_{oo}$ salinity and at temperatures between 3° and 13° C. These represent average salinity and minimum and maximum temperatures to be expected in the area (7). It was not practical to try to transport the needed quantities of natural sea water from the coast to Fairbanks, nor were storage facilities for sea water available. Artificial sea water offered the further advantage of being uncontaminated by plankton, whose metabolic activities would have added unknown and probably unmeasurable factors to the experiments.

The choice of experimental animals was dictated by a number of factors. Pragmatically, those species most valuable to the fisheries should be the first choice, and here the several species of Pacific salmon (genus Oncorhynchus) lead all the rest. Among the salmon, the chum (O. keta) and the sockeye (O. nerka) offered a number of advantages. In particular, the presumed availability of sockeye from the hatcheries of the Alaska Department of Fish and Game made this species a logical choice. Chums are not cultured in Alaska. Unfortunately, when the project finally got under way, sockeye were not available. We turned to the coho salmon (O. kisutch) which are rather more difficult to acclimate to salt water, but which were readily obtainable at the time. Later, when

sockeye were once again available, some experiments were run with this species.

The study was established in two phases. The first involved exposure of experimental fish to various amounts of crude oil from the Prudhoe Bay field in northern Alaska, while in the second phase the fish were exposed to various individual components of crude oil.

OBJECTIVES

Phase I was designed to determine the degree to which crude oil would affect fishes and to show the mechanisms by which the effects were brought about. Phase II attempted to do the same thing for several classes of hydrocarbons which are normally found in crude oil, thus hopefully identifying the particular groups of compounds responsible for the effects observed in Phase I.

As a result of some suggestive data accumulated in the first part of this study, we had planned to analyze swim bladder gases during the second phase of investigations. Unfortunately, the fish that were available during the second stage were much too small to allow this. Indeed, most of the experimental fish of the second phase were so small that blood analyses had to be curtailed. We could not obtain enough blood from single specimens to run complete analyses.

SECTION IV

MATERIALS AND METHODS

GENERAL

Young coho (Oncorhynchus kisutch) and sockeye (O. nerka) salmon were obtained from the Fire Lake Hatchery of the Alaska Department of Fish and Game. These fish were derived from local Alaskan stocks, the coho from Ship Creek, near Anchorage, and the sockeye from Bear Creek, near Seward. At the hatchery, the fish were placed in plastic bags of water, the bags sealed and put in styrofoam shipping boxes. They were then trucked to the Anchorage airport (about 20 miles [32 km]) and sent by air freight to Fairbanks. Except for one shipment, mortalities due to this handling were always less than 1%. The exceptional shipment had apparently been left on a loading dock in full sunshine for about six hours. More than half the fish in that shipment were dead on arrival at Fairbanks.

At the laboratory, the fish were put into two large circular tanks, each containing approximately 1135 liters of conditioned fresh water. The water in these tanks was kept at 8° C and circulated by a Min-O-Kool chiller-circulator. Filtration was done by pumping the water through a home-made fiberglass-charcoal filter with a small submersible pump. Waste material was removed once or twice a day. Fish in the stock tanks were fed five days a week with Oregon fish pellets.

After the fish had become acclimated to the stock tanks (usually two to four days, judged by their behavior in the tanks), Instant Ocean brand artificial sea salts were added over a period of about a week to bring the salinity up to approximately 30 °/oo. The fish were allowed to acclimate to the salt water for about another week before being used in experiments. Time of acclimation varied between 3 and 10 days, depending on size and species. Sockeye salmon adapted much more readily than did coho, and larger individuals, especially those beginning to "smolt up," adapted more easily than did smaller ones.

Experiments were conducted in Instant Ocean brand aquaria of 25 gallon (95 liter) dapacity, each containing 73 liters of conditioned salt water from the stock tanks. The gravel-like filtration material that is a standard part of these aquaria was not used in these experiments, as it would have been impossible to clean after being fouled with the experimental substances. The squaria were fully aerated and each was covered with a sheet of glass. Details of experimental procedures are given below.

PHASE I

Five series of experiments were done on coho salmon. With one exception (the final experiment of this phase), analyses were done after 96 hours. In Series I, measured amounts of crude oil were poured on the surface of the water. Behavior of the fish was observed, mortalities recorded, blood parameters (Na^+, K^+, Cl^-, CO_2, O_2, pH, serum proteins) analyzed, tissue samples (gill stomach, pyloric caeca, intestine, liver, fatty tissue, kidney, spleen) removed and fixed in Bouin's fluid. Experiments and controls were replicated at 3°, 8°, and 13° C. Environmental parameters monitored were O_2, CO_2, pH, H_2S, NH_3, salinity, copper, zinc, iron, and lead. Dead fish were removed daily.

For the blood parameters, serum Na^+ and whole blood K^+ were determined with a Coleman model 6-20 flame spectrophotometer. Serum Cl^- was titrated with an Oxford microtitrometer, following the Oxford modification of the method of Schales and Schales (6). CO_2, O_2, and pH were measured with a Corning model 16 blood pH/gas analyzer. Serum proteins were examined by disc gel electrophoresis with a Canalco model 1200 electrophoresis apparatus with scanner and printer.

Among the environmental parameters, salinity was determined with a Beckman model RS7-B salinometer; pH was read on a Coleman model 38A pH meter; oxygen was measured by the modified Winkler method and CO_2 was calculated from pH values of acidified samples (8). H_2S, NH_3, and heavy metals were determined by personnel of the Arctic Environmental Research Laboratory, Environmental Protection Agency.

Series II was the same as Series I, except that the oil was physically mixed in each tank by means of a stream of water (approximately 19 liters/minute) for five minutes at the beginning of each experiment.

In both Series I and II, the fish to be analyzed were anesthetized with MS 222 (Tricaine methane sulfonate). After trying narious methods, we concluded that the most satisfactory way of obtaining blood was to cut off the animal's tail and take blood from the caudal aorta. This method was used throughout the study.

In Series III, fish were anesthetized with MS 222, then dipped into a film of crude oil to simulate leaping or swimming through an oil spill. The fish were then placed in tanks of clean, aerated water, and excess oil carried into the tanks on the bodies of the fish was skimmed off the surface of the water. Mortalities were recorded and specimens were sacrificed at intervals for study of gill tissues. Controls were treated similarly, except that they were dipped into clean water instead of oil.

Experimental procedure of Series IV consisted of placing one drop of crude oil directly on the first gill on each side of an anesthetized

fish. The fish were then placed in tanks of clean water. Data were recorded as for Series III.

In Series V, crude oil in amount of 1g/100g body weight was forced gently into the stomach of each anesthetized fish from a syringe, The fish were put into tanks of clean salt water and observed, Experimental animals were examined for blood chemistry and histology of gut tissues. Controls were treated in the same way, but received salt water instead of oil.

The same five series of experiments were performed on sockeye salmon.

In all series, statistical significance was determined from 2x2 contingency tables (1, 3).

PHASE II

This phase of the work involved testing various crude oil components for toxicity. For reasons given in the discussion section, environmental parameters were not monitored, tissues were not taken, and only those blood parameters were examined which had appeared significant in earlier experiments. All experiments were conducted with coho salmon. Average weight of fish ranged from about 5 grams each in the fall of the year to nearly 40 grams in May. The number of fish per tank was adjusted to provide a ratio of 1 gram or less of fish per liter of water.

After the fish had been acclimated, as in Phase I, various amounts of test substance were introduced into the tanks, simply by squirting measured volumes from a small syringe. Basic quantities of substance were the equivalents of 1, 10, and 100 parts per million, but amounts varied as occasion demanded. Thus, 100 ppm xylene killed all the fish so quickly that no analyses could be made. Hence, for blood analyses after exposure to xylene, a concentration of 30 ppm was used.

The following substances were tested:

1. Pentane
2. Hexane
3. Heptane
4. Octane
5. 2-hexene
6. Cyclopentane
7. Ethylcyclopentane
8. Cyclohexane
9. Ethylcyclohexane
10. Cyclopentene
11. Cyclohexene
12. 1,3 cyclohexadiene
13. Benzene

14. Ethylbenzene
15. Xylene (standard laboratory mixture of isomers)
16. Toluene

As noted in the discussion of Phase I (see page 9), early analyses of blood chemistry produced inconclusive results. It was not until the experiment of 8-12 November 1971 that we realized that we had been analyzing survivors whose blood had returned more or less to normal. Hence, in Phase II, blood analyses were generally done every hour up to 4 hours and at 24 hours after exposure to the test substances. Because of the small size of the fish, it was usually impossible to obtain enough blood from a single specimen to analyze for all three ions (Na^+, K^+, and Cl^-), so tests were usually run for sodium and potassium or for sodium and chloride. Analyses were confined to those experiments in which significant mortalities of behavioral abnormalities were observed, specifically, benzene, toluene, xylene, and cyclohexene. The experiment with 1,3 cyclohexadiene was limited to observations of mortality and behavior.

SECTION V

RESULTS

PHASE I

Environmental Parameters

Salinity varied, apparently more or less at random, by about ± 0.1 °/oo, probably because of splatter and evaporation. pH remained constant at 8.1. Oxygen saturation ranged between 65% and 80%. There was no detectible free CO_2. Heavy metals, H_2S, and NH_3 did not vary from the traces found in the original tap water used to make the artificial sea water. In view of the constancy of these parameters, they were not monitored in Phase II.

Blood Parameters

Analyses of blood ions and pH made during Phase I produced only inconclusive results. There were indications of rises in Na^+, K^+, and Cl^-, and a suggestion of a slight decline in pH. In the final experiment of this phase, analyses were made at 24-hour intervals instead of at the end of the 96-hour period. These showed a rise in chloride levels during the first 24 hours, followed by a gradual drop back to normal levels in the next 2 or 3 days (Table 1). This discovery indicated a change in approach to more frequent analyses, which was put into effect in Phase II.

Apparently significant changes in some blood serum proteins were found in sockeye salmon. Sturdevant (9) reported that the bands designated by him as 9 and 17 tended to increase in density after exposure to crude oil.

Tissue Studies

More than 2,000 slides of the previously listed tissues were examined. No abnormalities were discovered that could definitely be ascribed to the experimental conditions. Clubbing of gill filaments was the most common anomaly, but this condition may be caused by any of several factors, such as accumulation of nitrogenous compounds in the water (J. Wallis, Superintendent, Fire Lake Hatchery, pers. comm.), myxobacterial infection (2), or insufficient pantothenic acid in the diet (5). It may be that metabolic wastes accumulated under the oil film, that the action of myxobacteria already present on the gills was enhanced by the experimental conditions, or that starvation of the fish during the course of the experiment resulted in a dietary deficiency. None of these seems probable, and, as some clubbing was present in the controls, we are without an explanation.

Table 1. CHANGES IN SERUM CHLORIDE OF YOUNG SOCKEYE SALMON EXPOSED TO VARIOUS AMOUNTS OF CRUDE OIL AT 8° C

Length of exposure, hrs	Av. Cl^- mEq/l	Std. dev.	n
	3500 ppm oil		
24	142.85	18.9865	6
48	150.16	19.1951	6
72	124.17	-	2
96	125.08	-	3
	1750 ppm oil		
24	162.43	22.6596	5
48	147.68	18.0647	5
72	140.27	16.2246	9
96	-	-	0
	500 ppm oil		
24	-	-	0
48	150.82	16.4617	8
72	138.80	18.1560	5
96	126.85	10.6888	6
	Control		
	126.95	8.7983	29

A few slides of stomach tissue from fish that had been force-fed with crude oil seemed to have thinner mucosa layers than did controls.

No other tissue abnormalities were noted.

Mortalities

Series I

Coho salmon-Experiment I-C-1, begun on 4 December 1970, involved exposing fish to 3500 ppm oil at each of three different temperatures. Mortalities after 72 hours are shown in Table 2. During the following 24 hours, the temperature controls on the 3° tanks failed, apparently because of leakage of refrigerant. Temperatures in these tanks varied during the night by as much as 7° C, and all fish in them died. For the remaining tanks, 96 hour mortalities are listed in Table 3.

Experiment I-C-2 began on 4 January 1971. It was the same as the previous experiment, but without the 3° C tanks. The oil used was a second aliquot of the original crude oil sample, and had been kept in a sealed five-gallon can, with considerable air space above the surface of the oil, for a month. After 96 hours, 12.5% of the 13° C experimentals and 12% of the 8° C experimentals had died, compared with 10% of the 13° C controls and none of the 8° C controls. The experimental death rate was not significantly different from that of the controls (Table 4).

A new sample of oil was obtained and experiment I-C-3 was begun on 12 February 1971. This experiment used 3500 ppm of oil at 8° C. Results are listed in Table 5.

Sockeye salmon-Sockeye salmon became available in Late June 1971. Experiment I-S-1 was begun on 8 July 1971. All tanks were held at 8° C. The various amounts of oil per tank and the resulting mortalities are shown in Table 6.

Experiment I-S-2, begun on 30 July 1971, was a repetition of I-S-1, but at temperatures of 3°-5° C. Some of the tanks would still not hold these low temperatures, hence the number of tanks involved was less than in previous experiments. Results are shown in Table 7.

Experiment I-S-3, begun on 12 August 1971, was again a repetition of I-S-1, but with all tanks at 13° C. Results are listed in Table 8.

Series II

Coho salmon-In experiment II-C-1, conditions were the same as in Series I, except that in half the tanks the oil was physically mixed with the water. Mortalities are given in Table 9.

Table 2. 72 HOUR MORTALITIES IN YOUNG COHO SALMON EXPOSED TO CRUDE OIL IN EXPERIMENT I-C-1

Tank	Temp. °C	Oil Conc. ppm equiv.	72 hour mortality	Average mortality	P
3	3	control	0 of 10	0%	
7	3	3500	2 of 10		
8	3	3500	7 of 10	45%	< 0.05
4	8	control	1 of 10		
5	8	control	0 of 10		
17	8	control	2 of 10	10%	
1	8	3500	7 of 10		
2	8	3500	9 of 10		
9	8	3500	0 of 10		
11	8	3500	0 of 10		
13	8	3500	5 of 10	42%	< 0.02
10	13	control	0 of 10	0%	
12	13	3500	8 of 10		
14	13	3500	3 of 10		
15	13	3500	1 of 10		
18	13	3500	8 of 10	50%	0.02

Table 3. 96 HOUR MORTALITIES IN YOUNG COHO SALMON EXPOSED TO CRUDE OIL IN EXPERIMENT I-C-1

Tank	Temp °C	Oil conc. ppm equiv.	96 hour mortality	Average mortality	P
4	8	control	1 of 10		
5	8	control	0 of 10		
17	8	control	1 of 10	10%	
1	8	3500	10 of 10		
2	8	3500	10 of 10		
9	8	3500	1 of 10		
11	8	3500	0 of 10		
13	8	3500	7 of 10	56%	<0.02
10	13	control	0 of 10	0%	
12	13	3500	9 of 10		
14	13	3500	3 of 10		
15	13	3500	3 of 10		
18	13	3500	10 of 10	62.5%	<0.02

Table 4. MORTALITIES OBSERVED IN YOUNG COHO SALMON EXPOSED TO 3500 PPM OF WEATHERED CRUDE OIL IN EXPERIMENT I-C-2

Temp. °C	Number of fish	Number and % dead after			
		24 hrs	48 hrs	72 hrs	96 hrs
Exptls.					
8	50	1 (2%)	4 (8%)	6 (12%)	6 (12%)
13	40	4 (10%)	4 (10%)	5 (12.5%)	5 (12.5%)
Controls					
8	10	0	0	0	0
13	10	0	0	1 (10%)	1 (10%)

Table 5. 96 HOUR MORTALITIES IN YOUNG COHO SALMON EXPOSED TO CRUDE OIL AT 8° C IN EXPERIMENT I-C-3

Tank	Oil Conc., ppm	96 hour mortality	Average mortality	P
2	control	1 of 11	9.1%	
7	3500	7 of 7		
8	3500	8 of 8		
17	3500	2 of 6		
18	3500	7 of 9		
4	3500	9 of 11	80.5%	< 0.02

Table 6. 96 HOUR MORTALITIES IN YOUNG SOCKEYE SALMON EXPOSED TO DIFFERENT AMOUNTS OF CRUDE OIL AT 8° C IN EXPERIMENT I-S-1

Tank	Oil Conc., ppm	96 hour mortality	Average mortality	P
7	control	1 of 10		
8	control	1 of 10	10%	
1	500	6 of 9		
2	500	5 of 9		
18	500	0 of 10	39.3%	>0.05, <0.10
9	1000	10 of 10		
10	1000	0 of 9		
11	1000	3 of 10	44.8%	<0.05
12	1750	0 of 10		
14	1750	1 of 10		
15	1750	1 of 10	6.7%	>0.10
4	3500	1 of 10		
16	3500	10 of 10		
17	3500	2 of 10	40%	<0.05

Table 7. 96 HOUR MORTALITIES IN YOUNG SOCKEYE SALMON EXPOSED TO DIFFERENT AMOUNTS OF CRUDE OIL AT 3°-5° C IN EXPERIMENT I-S-2

Tank	Oil Conc., ppm	96 hour mortality	Average mortality	P
17	control	0 of 10		
18	control	0 of 10	0%	
1	500	7 of 10		
2	500	4 of 10	55%	<0.01
11	1000	10 of 10		
12	1000	10 of 10	100%	<0.01
7	1750	10 of 10		
8	1750	10 of 10	100%	<0.01
14	3500	10 of 10		
15	3500	8 of 10	90%	<0.01

Table 8. 96 HOUR MORTALITIES IN YOUNG SOCKEYE SALMON EXPOSED TO DIFFERENT AMOUNTS OF CRUDE OIL AT 13° C IN EXPERIMENT I-S-3

Tank	Oil Conc., ppm	96 hour mortality	Average mortality	P
1	control	0 of 10		
4	control	0 of 10		
8	control	0 of 10		
9	control	0 of 10	0%	
11	500	0 of 10		
13	500	0 of 10	0%	>0.10
7	1000	1 of 9		
14	1000	0 of 10		
15	1000	1 of 10	6.9%	>0.10
16	1750	0 of 10		
17	1750	1 of 10	5%	>0.10
2	3500	5 of 10		
5	3500	1 of 10		
18	3500	0 of 10	20%	0.02

Table 9. 96 HOUR MORTALITIES IN YOUNG COHO SALMON EXPOSED TO MIXED OR UNMIXED CRUDE OIL AT 8° C IN EXPERIMENT II-C-1

	Oil Conc., ppm	Number of fish	Number and % dead
Unmixed Series			
	0	5	0
	10	5	0
	100	5	4 (80%)
	300	5	3 (60%)
	625	5	0
	1250	5	0
	2500	5	1 (20%)
Mixed Series			
	0	5	0
	10	5	0
	100	5	0
	300	5	0
	625	5	0
	1250	5	0
	2500	5	3 (60%)

Series III and IV

The results of these experiments are listed in Table 10. Although there was some mortality among the experimentals, it was not statistically significant.

Series V

In the ingestion experiments of Series V, the mortalities in the experimental series did not differ significantly from those of controls.

Behavior

The young salmon of both species, when subjected to direct exposure to crude oil (Series I and II) began to show signs of stress, or at least abnormal behavior, in as little as 45 minutes after the oil had been introduced into the tanks. The first indication was that all fish under oil slicks of 500 ppm equivalent or greater began to swim at the surface of the water with their dorsal fins and the upper lobes of the caudal fins in the oil film.

A second indication of stress was a loss of equilibrium. This generally began to appear within 24 hours. The salmon tilted to one side and their swimming became weak. Subsequently, affected individuals assumed a head-up, tail-down position, which eventually might become vertical. All individuals did not show these symptoms. Of those that reached the vertical position, only a very few recovered. Salmon that did recover have been kept under oil films for as long as 30 days. These experiments involved a single application of oil; presumably most of the volatiles had disappeared by the time the fish recovered.

Salmon of both species that had been force-fed with oil showed no abnormal behavior except that, within 2 hours of recovery from anesthesia, they began to excrete large amounts of oil and mucus. This continued for up to 12 hours, by which time it is assumed that all the oil had passed through the intestinal tract.

Fish in the experiments of Series III and IV showed no noticeable abnormal behavior after recovery from anesthesia.

PHASE II

Mortalities

The following did not produce significant mortalities when applied in amounts up to 100 ppm equivalent:

Pentane
Hexane

Table 10. MORTALITIES OBSERVED IN EXPERIMENTS III-C-1, IV-C-1, III-S-1, and IV-S-1

Tank	Condition*	Number alive after hrs 0	24	48	72	96
Experiments III-C-1 and IV-C-1						
4	Dip	5	4	4	4	4
17	Dip	5	5	5	5	5
16	Dip	5	4	4	4	4
14	Drop	5	5	5	5	5
15	Drop	5	5	5	5	5
12	Control	6	6	6	6	6
Experiments III-S-1 and IV-S-1						
1	13° Dip	10	10	8	8	7
2	13° Drop	10	10	9	9	9
4	13° Control	11	11	11	11	11
15	8° Dip	10	10	10	10	9
16	8° Drop	10	10	9	9	9
17	8° Control	10	10	10	10	10

*Dip: fish dipped into a thick film of oil, then placed in a tank of clean water.

Drop: one drop of crude oil placed directly on first gill of each side, fish then placed in tank of clean water.

Heptane
Octane
Cyclopentane
Ethylcyclopentane
Cyclohexane
Ethylcyclohexane
Cyclopentene
Cyclohexene

Significant mortalities were produced with all of the following:

1,3 cyclohexadiene
Benzene
Ethylbenzene
Xylene
Toluene

The results for the last five compounds are listed in Tables 11 through 15.

Behavior

The behavior of fish treated with the various pure substances was, in general, like a speeded-up and exaggerated version of the behavior under crude oil.

Pentane, hexane, heptane, and octane produced the least pronounced reactions. No abnormalities were observed at low concentrations, but when 100 ppm of these substances were applied, the fish showed signs of mild irritation. These consisted of rather rapid, erratic movement, and "coughing." In the latter, the fish opened their mouths and "backed water" with their pectoral fins, meanwhile holding their bodies straight. When 500 and 1000 ppm were used, the same symptoms were more pronounced and persisted for up to 72 hours.

No noticeable alterations of behavior were found in the experiments with 2-hexene, cyclopentane, ethylcyclopentane, cyclohexane, and ethylcyclohexane.

With cyclopentene, the increased activity and "coughing" appeared within 10 minutes of application of 50 and 100 ppm equivalents. These phenomena were much reduced after 2 hours and had disappeared by 4 hours after initial exposure.

The reactions of the fish to 100 ppm of cyclohexene were very different from the reactions to any other substance tested. There was no sign of erratic swimming or of "coughing." Instead, within 30 minutes of application of the cyclohexene, the fish began to undergo "spasms." All the fins were fully spread, the body appeared to become rigid, and the fish quivered for several seconds. Upon relaxation, the fish swam normally for several minutes, then "spasmed" again. This went on for 3 to 4 hours, after which the fish once again seemed entirely normal.

Table 11. MORTALITIES PRODUCED WITH YOUNG COHO SALMON AND 50 PPM OF 1, 3 CYCLOHEXADIENE AT 8° C

Tank		Number alive after hrs 0	24	48	72	96	% mortality	P
5	control	3	3	3	3	3		
6	control	3	3	3	3	3		
9	control	3	3	3	3	3	0	
17		3	2	2	2	2		
1		3	2	2	2	2		
2		3	2	2	2	2		
4		3	3	3	3	3		
8		3	3	3	3	3		
7		3	2	2	2	2		
11		3	1	1	1	1		
10		3	1	1	1	1		
12		3	2	2	2	2		
18		3	2	2	2	2	33.3	<0.005

Table 12. MORTALITIES PRODUCED WITH YOUNG COHO SALMON AND BENZENE AT 8° C

Tank	Conc. ppm	Number alive after hrs 0	24	48	72	96	% mortality	P
16	control	10	10	10	9	9		
17	control	10	10	10	10	10		
18	control	10	10	10	10	10	3.3	
12	1	10	9	9	9	9		
14	1	10	10	10	10	10		
15	1	10	10	10	10	10	3.3	>0.05
1	10	10	10	10	10	10		
2	10	10	10	10	10	9		
4	10	10	10	10	10	10	3.3	>0.05
10	50	10	3	3	3	3		
11	50	10	6	5	5	5	60	<0.005
7	100	10	0	0	0	0		
8	100	10	0	0	0	0		
9	100	10	0	0	0	0	100	<0.005

Table 13. MORTALITIES PRODUCED WITH YOUNG COHO SALMON AND ETHYL-BENZENE AT 8° C

Tank	Conc. ppm	Number alive after hrs 0	24	48	72	96	% mortality	P
18	control	10	10	9	9	9		
13	control	10	9	9	9	9		
16	control	10	10	10	10	10	6.7	
15	10	10	9	9	8	8		
14	10	10	9	9	9	8		
12	10	10	10	10	10	10	13.3	>0.05
11	50	10	0	0	0	0		
10	50	10	0	0	0	0		
8	50	10	0	0	0	0	100	<0.005

Table 14. MORTALITIES PRODUCED WITH YOUNG COHO SALMON AND XYLENE AT 8° C

Tank	Conc. ppm	Number alive after hrs. 0	24	48	72	96	% mortality	P
8	control	10	9	9	9	8		
14	control	10	10	9	9	8		
18	control	10	10	8	7	7	23.3	
4	1	10	10	9	9	8		
7	1	10	10	10	9	9		
15	1	10	9	7	6	6	23.3	>0.05
9	10	10	8	7	7	7		
12	10	10	9	8	8	7		
16	10	10	10	9	7	7	30	>0.05
10	100	10	0	0	0	0		
11	100	10	0	0	0	0		
17	100	10	0	0	0	0	100	<0.005

Table 15. MORTALITIES PRODUCED WITH YOUNG COHO SALMON AND TOLUENE AT 8° C

Tank	Conc. ppm	Number alive after hrs 0	24	48	72	96	% mortality	P
16	control	10	9	9	7	7		
17	control	10	10	10	10	10		
18	control	10	9	9	9	9	13.3	
12	1	10	10	10	9	9		
14	1	10	9	9	9	9		
15	1	10	9	9	8	8	13.3	>0.05
8	10	10	10	10	9	9		
9	10	10	10	10	10	8		
11	10	10	10	10	10	10	10	>0.05
7	50	10	1	0	0	0	100	<0.005
1	100	10	1	0	0	0		
2	100	10	0	0	0	0		
4	100	10	1	0	0	0	100	<0.005

At 50 ppm, also, a few fish "spasmed," but the reactions were not so severe and disappeared in less than 2 hours.

The remaining test substances, 1,3 cyclohexadiene, benzene, ethylbenzene, xylene, and toluene, at 20 to 100 ppm, all produced what appeared to be a single set of behavioral abnormalities. These began, within 15 to 20 minutes of application, with rapid, violent, and erratic swimming. The fish dashed about the aquaria, leaping out of the water, banging against the sides and covers of the tanks. This was followed by "coughing," loss of equilibrium, and death. Fish that survived the first few hours of exposure generally survived to the end of the experiment (see Tables 11-15).

Blood Chemistry

Results of the blood analyses are listed in tables 16-20. In almost every analysis, there is a distinct initial rise in the concentration of the ion concerned, followed by a decline to about the level observed in the controls. This pattern is most obvious with sodium, less so with potassium and chloride.

Table 16. CHANGES IN BLOOD SODIUM AND CHLORIDE ION OF YOUNG COHO SALMON AFTER EXPOSURE TO 100 PPM CYCLOHEXENE AT 8° C

Time	$\bar{x}$ mEq/l	s	n
	Sodium		
control	175.19	11.8912	18
1 hour	194.33	31.0648	20
2 hours	173.25	15.3357	20
3 hours	170.25	16.4094	20
	Chloride		
control	133.94	6.9806	16
1 hour	164.00	8.5599	12
2 hours	153.89	5.1735	19
3 hours	131.14	6.8529	14

Table 17. CHANGES IN BLOOD SODIUM AND POTASSIUM OF YOUNG COHO SALMON AFTER EXPOSURE TO 15 PPM BENZENE AT 8° C

Time	$\bar{x}$ mEq/l	s	n	
		Sodium		
control	133.81	5.4920	16	
1 hour	160.24	24.8600	16	
2 hours	157.36	7.6405	16	
3 hours	160.33	17.8384	16	
4 hours	189.58	24.6917	14	+ 2 off top of scale
24 hours	153.68	29.8161	14	
		Potassium		
control	4.34	0.3543	16	
1 hour	4.81	0.5040	16	
2 hours	4.70	0.8788	16	
3 hours	5.02	0.8312	16	
4 hours	4.01	0.3708	16	
24 hours	3.87	0.4451	14	

Table 18. CHANGES IN BLOOD SODIUM AND POTASSIUM OF YOUNG COHO SALMON AFTER EXPOSURE TO 30 PPM XYLENE AT 8° C

Time	$\bar{x}$ mEq/l	s	n
		Sodium	
control	229.17	32.9875	14 + 2 off top of scale
1 hour	233.22	14.2992	16
2 hours	212.98	22.6155	14 + 2 clotted
3 hours	200.11	11.2735	16
4 hours	248.34	17.1728	8
24 hours	177.09	16.4858	16
		Potassium	
control	4.43	0.9013	16
1 hour	4.57	0.5167	16
2 hours	3.70	0.6631	16
3 hours	4.62	0.9524	16
4 hours	4.84	1.0422	8
24 hours	3.96	1.3707	16

Table 19. CHANGES IN BLOOD SODIUM AND CHLORIDE OF YOUNG COHO SALMON AFTER EXPOSURE TO 30 PPM XYLENE AT 8° C

Time	$\bar{x}$ mEq/1	s	n
		Sodium	
control	186.39	18.5469	18
1 hour	248.86	13.2331	18
3 hours	210.08	16.8551	20
5 hours	197.25	24.7972	20
		Chloride	
control	134.47	9.2398	19
1 hour	134.75	9.9028	16
3 hours	137.10	10.3004	10
5 hours	140.06	5.6858	16

Table 20. CHANGES IN BLOOD SODIUM AND POTASSIUM OF YOUNG COHO SALMON AFTER EXPOSURE TO 30 PPM TOLUENE AT 8° C

Time	$\bar{x}$ mEq/l	s	n
		Sodium	
control	208.19	37.6502	12 + 2 off top of scale
1 hour	624.60	11.3566	4 + 12 off top of scale
2 hours	225.84	26.4360	8 + 2 off top of scale
3 hours	215.84	47.9977	10 + 6 off top of scale
4 hours	220.01	44.3942	8 + 8 off top of scale
		Potassium	
control	4.06	0.7699	16
1 hour	4.75	1.0571	16
2 hours	5.83	0.5223	10
3 hours	5.11	0.9596	14
4 hours	4.89	0.8110	16

SECTION VI

DISCUSSION

Sturdevant's (9) results with serum proteins appear to be clear-cut. Bands 9 and 17 on the electrophoresis gel both increased in density after exposure to crude oil. Band 9 lay in a position corresponding to the hemoglobin region of human serum, while band 17 lay in the albumin region. Despite a rather high degree of variation in the data, which he ascribed to various factors such as individual variation in the test animals, age and condition of reagents, and operator inconsistence, Sturdevant was reasonably certain of the following:

1. Serum proteins of sockeye salmon responded to exposure of the animal to crude oil.
2. Length of exposure was significant in the effects produced on both bands.
3. Increased concentrations of oil produced increased effects on band 9 but not on band 17.

Sturdevant did not attempt to assess the physiological value of these effects, as he was unable to identify the proteins involved.

The mortalities observed in Phase I, wherein fish were exposed to Prudhoe Bay crude oil, show that this oil is highly toxic. Quantities of 500 ppm equivalent, or greater, produced mortalities that were statistically significant when compared to mortalities of controls. This concentration could easily occur if spilled oil were swept into a shallow cove, for example. Whether or not free-living fish could and would escape from underneath such a slick is unknown.

The lack of significant mortality in experiment I-C-2 is of particular interest. The oil used in this experiment had been exposed for 30 days to the large volume of air in the container. It was remarked when the can was re-opened that the oil was much less odorous than it had been a month earlier. It is reasonable to assume that during this period most of the volatile toxic materials in the oil had evaporated, resulting in the negligible death rates of this experiment.

The experiments with sockeye salmon suggest that, at least for this species, deaths are inversely related to temperature. Several explanations are possible. One is that at the low temperatures (3°-5° C), the volatile substances evaporate more slowly, hence the fish are exposed to higher concentrations for a longer time. It is also possible that the fish are physiologically less able at low temperatures and hence succumb more readily to adverse conditions.

In experiment II-C-1, oil was poured in the surface of the water in half the experimental tanks, but was physically mixed with the water

in the remaining experimental tanks. The results of this experiment are peculiar. The mortalities in individual tanks wherein deaths occurred are significant, as compared with the controls. However, the overall mortality in all experimental tanks is not significant, and we have no explanation.

On the basis of our experimental results, the aromatic hydrocarbon toxicity increases in the following order: cyclohexane, cyclohexene, 1, 3 cyclohexadiene, toluene, xylene, benzene, and ethylbenzene. The first part of the series clearly correlates toxicity with the number of unsaturated bonds.

At the higher concentrations, most deaths occurred within the first few hours. However, at lower concentrations, fish that survived these first hours of exposure generally survived to the end of the experiment.

It seems fairly obvious that survival in the experiments of Phase II was related to the volatility of the test substance and its structure. A poly-unsaturated, six-carbon-atom ring seemed to be the toxic agent. The unsaturated cyclic hydrocarbons were the most toxic materials tested and were also the most volatile. A fish that could withstand the first few hours, when the concentration of test substance was highest, stood a good chance of surviving the experiment, for the volatile nature of the test substances resulted in rapidly decreasing concentrations as time went on.

The blood analyses done in Phase II generally show a rise in concentration of monovalent ions during the first few hours after exposure, followed by a return towards the control level. The mean values found in the experimental fish often are not significantly different from the control values. Nevertheless, the pattern is too consistent to be ignored. Likewise, few of the experimental values fall outside the ranges given by Holmes and Donaldson (4) for O. kisutch and related species. It is quite possible that the rapid, uncontrolled change in ion concentration, rather than the concentration per se, is the important factor.

The mechanism of toxicity is still not entirely clear, but the evidence leads to some reasonable speculations.

The behavior of the fish in response to oil and to pure substances is, in general, typical of the response of fishes to toxic hydrocarbons (C. Bond, Department of Fisheries and Wildlife, Oregon State University, pers. comm.). Swimming at the surface, with the dorsal and caudal fins touching the oil slick, is difficult to interpret. It is not a response to reduced light, for fish in tanks covered with heavy black paper retained the usual, more or less random distribution, while those in tanks covered with oil, but brightly illuminated from the side, swam at the surface. Likewise, it is not a reaction to insufficient

oxygen, for full aeration was maintained and dissolved oxygen was always between 65% and 80% saturation. However, other aspects of the observed behavior are more amenable to interpretation.

The fact that the most toxic materials tested are fat solvents suggests that the permeability of membranes, particularly in the gills, is increased through the loss of fatty substances. This idea is supported by the observed increases in the concentrations of the monovalent ions sodium, potassium, and chloride in the blood. Since our fish were tested in a hypertonic environment, increased intake of monovalent ions would be expected if the permeability of the gill membranes were increased. (Parenthetically, the ability of the fish to restore the fat balance in these tissues must be quite rapid. At any rate, blood ions approached normal concentrations almost as rapidly as the volatile test materials were lost from the tanks.)

The increased ion concentrations in the blood probably interfere with carbonate and pH adjustment. It is reasonable to assume that the chloride shift has been altered. This, in turn, would affect the CO_2-HCO_3^- balance and could interfere with the fish's ability to control the gas content of the swim bladder. Loss of this control could account for the loss of equilibrium. Loss of equilibrium, particularly the head-up, tail-down position, accompanied by weakened swimming movements, is a typical symptom of CO_2 poisoning (10). If the fish cannot control the CO_2-HCO_3^- balance in its blood, internal accumulation of CO_2 may be a contributing factor in producing death.

Very little is known of the symptoms of ionic imbalance in fishes, but by analogy with mammalian and especially human symptoms, it seems probable that this, too, is an important factor. In particular, muscular hypertension, which was noted in several experiments, and loss of muscular control, may be attributed to ionic imbalance.

In summary, then, the toxicity of crude oil to fishes is most likely attributable to unsaturated cyclic compounds in the oil. These compounds probably act by increasing the cell membrane permeability of the gills, resulting in ionic imbalance and internal CO_2 poisoning.

SECTION VII

REFERENCES

1. Bennet, B. M., and C. Horst. Supplement to Tables for Testing Significance in a 2x2 Contingency Table. Cambridge, England, Cambridge U. Press, 1966. 28 p.

2. Davis, H. S. Culture and Diseases of Game Fishes. Berkeley and Los Angeles, U. of California Press, 1961. p. 261-264; 281-282.

3. Finney, D. J., R. Latscha, B. M. Bennett, and P. Hsu. Tables for Testing Significance in a 2x2 Contingency Table. Cambridge, England, Cambridge U. Press, 1963. 103 p.

4. Holmes, W. N., and E. M. Donaldson. The Body Compartments and the Distribution of Electrolytes. In: Fish Physiology, Vol. 1, W. S. Hoar and D. J. Randall (eds.). New York and London, Academic Press, 1969. p. 1-89.

5. Rucker, R. R., H. E. Johnson, and G. M. Kaydas. An Interim Report on Gill Disease. Prog. Fish-Cult. 14(1):10-14, January 1952.

6. Schales, O., and S. S. Schales. A Simple and Accurate Method for the Determination of Chloride in Biological Fluids. J. Biol. Chem. 140(3):879-884, 1941.

7. Scripps Institution of Oceanography. Oceanic Observations of the Pacific: 1955. Berkeley and Los Angeles, U. of California Press, 1962.

8. Strickland, J. D. H., and T. R. Parsons. A Practical Handbook of Seawater Analysis. Bull. Fish. Res. Bd. Canada, Ottawa 167:21-33, 1968.

9. Sturdevant, D. C. The Influence of Crude Oil on the Serum Proteins of the Sockeye Salmon. Fairbanks, M.S. Thesis, U. of Alaska, May, 1972. 43 p.

10. van Duijn, C., Jr. Diseases of Fishes. 2 ed. Springfield, Charles C Thomas, 1967. p. 93-95.